

Sometimes Intermediates Do the Job!

The SARS coronavirus main proteinase is a prime target for antiviral therapy. In this issue of *Chemistry & Biology*, Wu et al. describe potent inhibition of the enzyme by benzotriazole esters, which were originally obtained as intermediates in the synthesis of lopinavir derivatives [1].

Since the discovery of HIV-1 proteinase in the mid-1980s, this enzyme probably has become the best-characterized peptidase, with several hundreds of crystal structures of inhibitor complexes determined to date. However, the main proteinase of SARS coronavirus (M^{pro} , also called 3CL pro) has a good chance to catch up. When the first three-dimensional structure of a coronavirus M^{pro} was published in 2002, that of the enzyme from transmissible gastroenteritis virus (TGEV) [2], interest in the coronaviridae was only moderate, since these viruses were considered relatively harmless to human health. This assessment changed dramatically in late March, 2003, when it was found that the ongoing global outbreak of the highly infectious severe acute respiratory syndrome was caused by a new coronavirus, subsequently named SARS-CoV. Since then, efforts to discover anti-SARS drugs have been numerous, in order to be prepared should the virus raise its ugly head again.

Coronavirus genomes code for two large polyproteins, pp1a and pp1ab, that are processed by viral proteinases to yield the individual components of the large replicase complex. Most coronaviruses have three cysteine proteinases that are responsible for this processing: two papain-like proteinases and the main proteinase, M^{pro} . Interestingly, one of the two papain-like proteinases is absent in the SARS coronavirus, and the other one has been shown to have the additional function of a deubiquitinating enzyme [3, 4]. While the papain-like proteinases together are only responsible for three cleavage reactions near the N terminus of the polyproteins, the M^{pro} cleaves these huge substrates (molecular masses of 450–750 kDa) at no less than 11 sites. Since this reaction is essential for viral replication, the main proteinase is obviously a prime target for interference by inhibitors [5].

As a first step toward inhibitor design for coronavirus M^{pro} s, the crystal structure was elucidated for a complex between the TGEV enzyme and a hexapeptidyl chloromethyl ketone inhibitor that had an amino acid sequence corresponding to the specificity of the enzyme [6]. The mode of binding of this inhibitor to the target enzyme was found to be related (although not identical) to what had been seen earlier in a complex between the rhinovirus (HRV-2) 3C proteinase and compound AG7088 (Figure 1A), a vinyllogous ethyl ester developed by Agouron Inc. (now a division of Pfizer) for the treatment of the common cold caused by rhinoviruses [7]. This observation led to the proposal that AG7088 should be a good starting point for the design of anti-SARS inhibitors [6].

The crystal structures of the main proteinases of human coronavirus 229E and then of the SARS virus itself were solved within weeks after identification of the new virus [6, 8]. The structural insight along with information on the flexibility of the enzyme [9] enabled researchers world-wide to use structure-based design [5] and virtual screening methods [10] to prepare new inhibitors of the SARS-CoV M^{pro} . Several derivatives of AG7088 (Figure 1B–D) have been described, which are much more active than the parent compound while displaying low toxicity in cell assays [11].

Originally, SARS patients in Hong Kong and Toronto were treated with ribavirin. When it surfaced that this compound is not efficient against the virus, at least not at nontoxic concentrations, HIV proteinase inhibitors were tested preclinically. Surprisingly, some but not all of these did inhibit the SARS-CoV main proteinase in vitro and in cell culture. Thus, Wong and co-workers found that lopinavir (Figure 1E) inhibited the M^{pro} with an IC_{50} of $\sim 50 \mu M$ [12].

The new contribution from the same group, presented in this issue [1], originated from an attempt to improve the inhibitory properties of lopinavir. The authors assembled a library of lopinavir-like compounds by coupling an amine or a 1,4-diamine with various acids in microtiter plates, followed by screening in situ. O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU) was used as a coupling reagent. Several lopinavir derivatives showed somewhat improved binding affinities, but it came as a big surprise that some of the intermediate benzotriazole esters resulting from the activation of the acid components by HBTU were nanomolar inhibitors of the M^{pro} ! Thus, we have the remarkable case here of a reaction intermediate being a highly active inhibitor of a target enzyme. Next, the authors synthesized a series of benzotriazole esters (Figure 1F) by condensation of HBTU with various aromatic carboxylic acids and found that compounds carrying electron-donating substituents were strong irreversible inhibitors of the M^{pro} , with a K_i of 21.0 nM for the best derivative. Using mass spectrometry, Wu et al. showed that the compounds acted by acylating the catalytic cysteine residue of the M^{pro} . Further, they also demonstrated that the benzotriazole derivatives were non-toxic to Vero E6 cells, the standard cells used in anti-SARS-CoV assays, at a concentration of 100 μM [1].

Yet, it is clear that these inhibitors are quite far from being drugs useable for treating coronavirus infections and SARS. Because of their mode of action, it seems likely that they will inhibit cellular cysteine proteinases as well as the viral one. However, these acylating compounds could potentially be modified by substituents occupying the S1 and S2 specificity pockets of the enzyme and are therefore useful starting points for designing more specific inhibitors.

As reactivity and specificity often counteract each other, it would be desirable to have inhibitors of the SARS-CoV main proteinase that do not covalently modify the catalytic cysteine residue. In their paper, Wu et al. [1] also describe benzotriazole ester surrogates that have

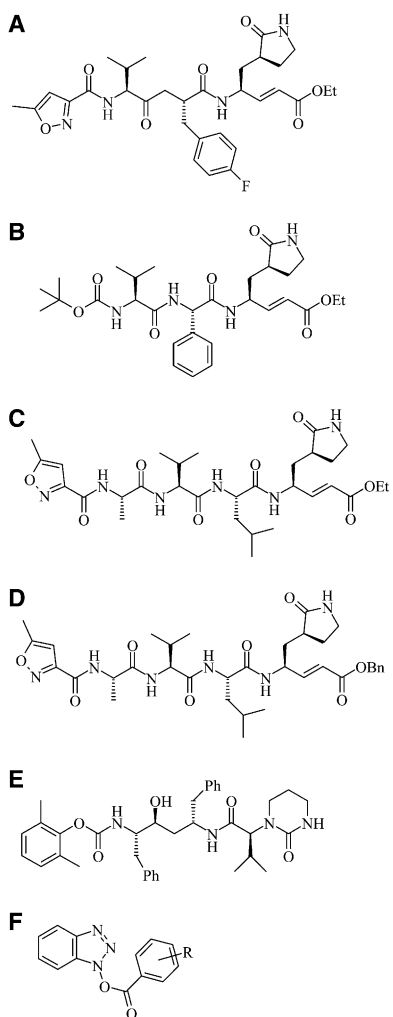


Figure 1. Chemical formulas of (A) AG7088, (B–D) AG7088 derivatives, (E) lopinavir, (F) 1-(benzoyloxy)-benzotriazole (R=4-NHCH₃, 4-N(CH₃)₂, or 4-N(C₂H₅)₂).

the ester oxygen replaced by a methylene group and are thus not susceptible to nucleophilic attack by the active site cysteine. Some of these compounds (such as 14, 17, and 18; see Scheme 3 of Wu et al. [1]) are competitive inhibitors with K_i values in the lower micromolar range and are thus among the most potent noncovalent inhibitors

of the SARS-CoV M^{pro} described to date. The discovery of compounds binding noncovalently to the M^{pro} may, in the end, constitute a more important milestone on the way to clinically useful inhibitors of the coronavirus main proteinase than identification of the acylating agents.

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Selected Reading

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Pharmacogenetics: Yeast Lead the Way

In this issue of *Chemistry & Biology*, Perlstein et al. [1] use genetically diverse strains of yeast to study the genetic basis of differences in cellular responses to

small molecules. Their results suggest that drug responses are regulated by a limited number of loci, and that this system can identify clusters of functionally similar molecules.

When given a standard dose of commonly prescribed drugs, a significant fraction of patients will either receive